

Resonance Rayleigh Scattering, Second-Order Scattering and Frequency Doubling Scattering Spectra of Copper(II)-Flutamide System with Anionic Surfactants and its Analytical Application

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Received: 11 July 2010 / Accepted: 29 December 2010 / Published online: 11 January 2011
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Abstract A simple, sensitive, and rapid method based on ion association, for the determination of FLD has been developed. Flutamide (FLD) can react with Cu(II) to form 1:1 cationic chelate at pH 2.2–7.0 McIlvaine buffer medium, which can further react with anionic surfactants (AS) such as sodium dodecyl sulfate (SDS), sodium lauryl sulfonate (SLS) and sodium dodecylbenzene sulfonate (SDBS) to form 1:1 ion-association complexes. As a result, the resonance Rayleigh scattering (RRS), second-order scattering (SOS) and frequency doubling scattering (FDS) were enhanced to the highest degree. The maximum RRS, SOS and FDS wavelengths of three ion-association complexes were located at 345/345 nm, 610/305 nm and 430/860 nm, respectively. The increments of scattering intensity (ΔI) were directly proportional to the concentration of FLD in certain ranges. The detection limits (3σ) of FLD for SDS, SLS and SDBS systems were 1.9 ng ml^{-1} , 2.1 ng ml^{-1} and 2.2 ng ml^{-1} (RRS method), 2.4 ng ml^{-1} , 2.7 ng ml^{-1} and 2.6 ng ml^{-1} (SOS method) and 2.3 ng ml^{-1} , 2.4 ng ml^{-1} and 2.5 ng ml^{-1} (FDS method), separately. The sensitivity of RRS method was higher than those of FDS and SOS methods. The optimum conditions of RRS method and the influence factors, the composition and the reaction mechanism have been discussed. Since the method is highly selective, it does not interference concomitant substances. These methods were applied successfully for the determination of FLD in pharmaceutical formulation and urine samples.

Keywords Flutamide (FLD) · Cu(II) · Resonance Rayleigh scattering · Anionic surfactants · Urine sample

Introduction

Flutamide (FLD), chemically known as 2-methyl-N-[4-nitro-3-trifluoromethyl] phenyl] propanamide (Fig. 1) is widely used as antineoplastic (hormonal) and antiandrogen drug [1]. It is a powerful nonsteroidal androgen antagonist which is used to treat prostate cancer, is believed to block androgen receptor sites. This drug and its primary hydroxy metabolite decrease metabolism of C-19 steroids by the cytochrome P-450 system at the target cells in the secondary sex organ [2]. It has been recently included in the United States Pharmacopoeia which involves a chromatographic method for the analysis of the pure drug and FLD capsules [3, 4]. The number of different methods have been reported for determination of the Flutamide in pharmaceutical formulation and biological fluids, including polarography, spectrophotometry, GC and HPLC [5–12].

In this work, we are reporting the impact of ternary ion-association complexes on RRS, SOS and FDS spectra. Because the sensitivity of RRS method is higher than those of SOS and FDS, the optimum conditions of the reactions and influence factors were mainly investigated by this method. We have found that Cu(II) reacted with FLD to form 1:1 cationic chelate $[\text{FLD}\cdot\text{Cu}]^+$, in the pH range 2.2–7.0 McIlvaine's buffer, at room temperature, which can result in a little enhancement of intensities of RRS, SOS and FDS. The $[\text{FLD}\cdot\text{Cu}]^+$ further reacted with anionic surfactants (AS) such as sodium lauryl sulfonate (SLS) and sodium-dodecylbenzene sulfonate (SDBS) to form 1:1 ternary ion-association complex $[\text{FLD}\cdot\text{Cu}(\text{II})](\text{AS})$. The intensities of

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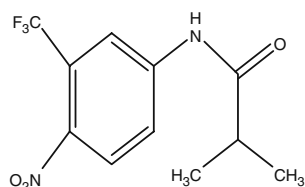


Fig. 1 Structure of flutamide (FLD)

RRS, SOS and FDS were substantially enhanced. Three ion association complexes have similar spectral characteristics and the maximum RRS, SOS and FDS wavelengths were located at 345/345 nm, 610/305 nm and 430/860 nm respectively. The increments of scattering intensity (ΔI) were directly proportional to the concentration of FLD in certain ranges. The effects of concomitant substance were tested and it showed that the RRS method has a good selectivity. A sensitive, simple, selective and rapid method was developed for the determination of FLD in pharmaceutical formulations. The mechanism for the ion-association reaction and the reason of RRS enhancement has also been discussed.

Experimental

Reagents

Standard solution of FLD was prepared by dissolving 0.0276 g FLD (Sigma Aldrich) in 100 ml (40:60) ethanol-water mixture. For working solution it was diluted to $25 \mu\text{gml}^{-1}$. The working solution of Cu(II) ($100 \mu\text{gml}^{-1}$) was prepared by dissolving 0.1 g of $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ which was diluted with water in 250 ml flask. Anionic surfactants solutions: SLS, SDS and SDBS (Sigma) of $1.0 \times 10^{-3}\text{M}$, $1.0 \times 10^{-3}\text{M}$ and $1.0 \times 10^{-2}\text{M}$, respectively, were prepared. Mclivaine's buffer solutions of different pH were prepared.

General Procedure

A 0.5 ml of AS, 0.5 ml of Cu(II) suitable amount of FLD solution and 1.0 ml of pH 5.0 Mclivaine's buffer were transferred into a 10 ml flask. The mixture was made up to the mark with water, shaken thoroughly and then left for 10 min. The RRS intensity (I_{RRS}) of the systems were recorded with synchronous scanning at $\lambda_{\text{ex}} = \lambda_{\text{em}}$. The SOS intensity (I_{SOS}) and the FDS intensity (I_{FDS}) of the systems were recorded at $\lambda_{\text{em}} = 2\lambda_{\text{ex}}$ and $2\lambda_{\text{em}} = \lambda_{\text{ex}}$, respectively. The I_{SOS} and I_{FDS} were plotted against different wavelengths to get SOS and FDS spectra. The scattering intensities (I_{RRS} , I_{SOS} and I_{FDS}) for the ion-association complexes and, I_0^{RRS} , I_0^{SOS} and I_0^{FDS} for the reagent blank were measured at their own maximum, $\Delta I_{\text{RRS}} = I_{\text{RRS}} - I_0^{\text{RRS}}$, $\Delta I_{\text{SOS}} = I_{\text{SOS}} - I_0^{\text{SOS}}$ and $\Delta I_{\text{FDS}} = I_{\text{FDS}} - I_0^{\text{FDS}}$.

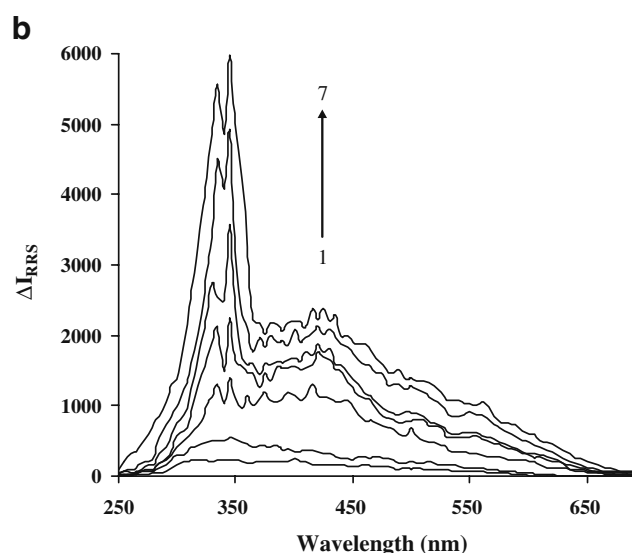
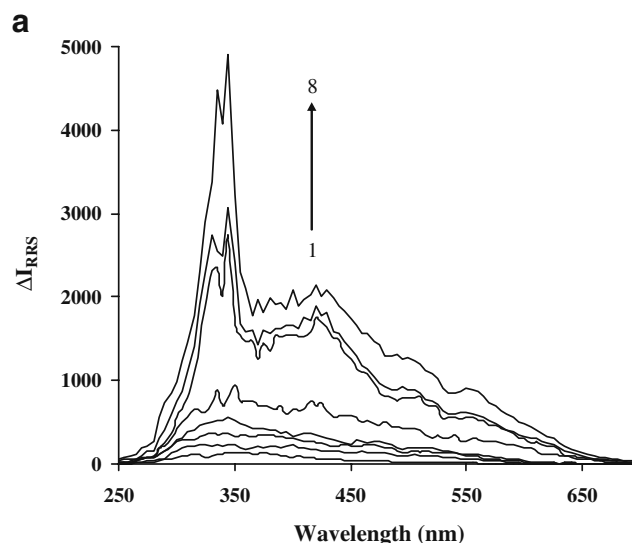


Fig. 2 (a) RRS spectra of FLD-Cu(II)-AS systems: From 1 to 8: (1) FLD, (2) Cu(II)-SDBS, (3) Cu(II)-SLS, (4) Cu(II)-SDS, (5) Cu(II)-FLD, (6) FLD-Cu(II)-SDBS, (7) FLD-Cu(II)-SLS and (8) FLD-Cu(II)-SDS. The concentration of FLD: $3.0 \mu\text{gml}^{-1}$; the concentration of Cu(II): $3.0 \mu\text{gml}^{-1}$; the concentration of SDS and SLS: $3.0 \times 10^{-4}\text{M}$; the concentration of SDBS: $4.0 \times 10^{-3}\text{M}$ (b) RRS spectra of FLD-Cu(II)-AS systems: From 1 to 7: the concentration of FLD: 0.5, 1.0, 2.0, 3.0, 4.0, 5.0 and $6.0 \mu\text{gml}^{-1}$

Results and Discussion

Spectral Characteristics

RRS Spectra

It can be seen (Fig. 2a) that the RRS intensities of AS, Cu(II) and FLD themselves are very weak. The binary chelate of Cu(II) with FLD exhibits only a minor change in the RRS spectra but in presence of anionic surfactants (SDS, SLS and SDBS) RRS intensities are markedly enhanced

due to the formation of ternary complex. All the reaction products have similar spectral characteristics as the maximum RRS intensity at 345 nm. The RRS intensity of the SDS system is highest among the three systems, followed by the SLS and SDBS. Figure 2b shows that the enhancement of RRS intensity for the FLD-Cu(II)-SDS system is directly proportional to the concentration of FLD. The RRS method was therefore more effective to the determination of FLD.

SOS Spectra

It has been observed that the SOS intensities similar to RRS intensities of AS, Cu(II) and FLD are very weak (Fig. 3a). Formation of the binary chelate shows a little change in the SOS spectra. It has been observed that when binary chelate further reacts with SDS, SLS, and SDBS to form ternary complexes SOS intensities are greatly enhanced. All the reaction products have similar spectral characteristics as the maximum SOS peaks are all the same at 610 nm. The decrease in scattering intensities (ΔI^{SOS}) of the system obeys the order SDS > SDBS > SLS. Taking FLD-Cu(II)-SDS as an example, when the λ_{ex} is at 610 nm, the changes of SOS intensities with different concentration of FLD were investigated. It can be seen from Fig. 3b that the enhancement of SOS intensity is linear to an increased concentration of FLD. The other two reaction systems under investigation followed the same pattern.

FDS Spectra

The intensity of FDS spectra of individual components in solution are not very strong but the binary chelates shows a little enhancement. When the binary chelate reacts with SDS, SLS and SDBS forming ternary complexes, FDS intensities are enhanced to a greater extent. The reaction products have similar spectral characteristics at λ_{max} 430 nm (Fig. 4a). The intensities of reaction products were different and their relative scattering intensities (ΔI^{FDS}) follows the order SDS > SLS > SDBS. When FDS Spectra of the system FLD-Cu(II)-SDS λ_{ex} at 430 nm was taken intensities of FDS spectra is proportional to the concentration of FLD (Fig. 4b). The sensitivity of RRS method is higher than those of SOS and FDS, therefore RRS method was apply to obtain optimum reaction condition.

Optimum Reaction Conditions

Effect of Acidity

The effect of different buffer solutions on RRS intensities were tested with Mclivaine's, Britson-Robinson and HAC-

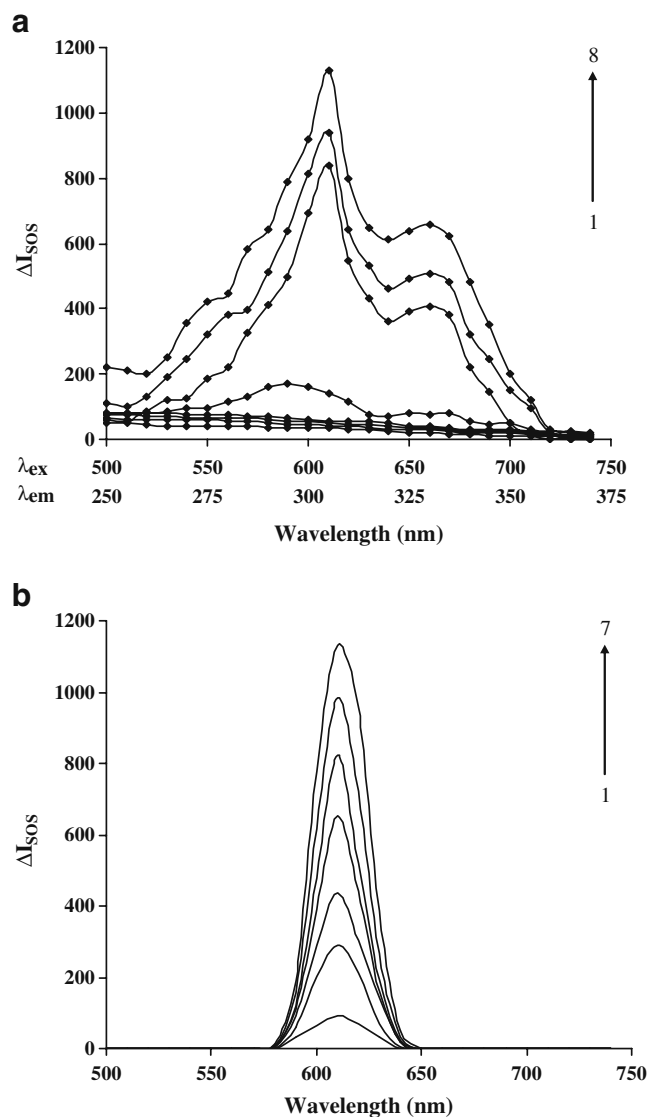


Fig. 3 (a) SOS spectra of FLD-Cu(II)-AS system: From 1 to 8: (1) FLD, (2) Cu(II)-SDBS, (3) Cu(II)-SLS, (4) Cu(II)-SDS, (5) Cu(II)-FLD, (6) FLD-Cu(II)-SDBS, (7) FLD-Cu(II)-SLS and (8) FLD-Cu(II)-SDS. The concentration of FLD: $2.0 \mu\text{gml}^{-1}$; the concentration of Cu (II): $2.0 \mu\text{gml}^{-1}$; the concentration of SDS and SLS: $3.0 \times 10^{-4} \text{M}$; the concentration of SDBS: $4.0 \times 10^{-5} \text{M}$ (b) SOS spectra of FLD-Cu(II)-AS system: From 1 to 7: the concentration of FLD: 0.3, 0.91, 2, 1.6, 2.0, 2.6, and $3.0 \mu\text{gml}^{-1}$

NaAc buffer and Mclivaine's buffer was found to be most suitable. Optimum pH range for the reaction was 2.2–7.0, 2.2–6.8 and 2.2–6.9 for SDS, SLS and SDBS systems, (Fig. 5) respectively. Thus, 1.0 ml of Mclivaine's buffer of pH 5.0 was taken in each case.

Effect of the Cu(II) Concentration

It can be seen (Fig. 6) that RRS intensity reached a maximum and then remained stable even when the concentration of Cu(II) was raised from 1.5 to $5.0 \mu\text{gml}^{-1}$.

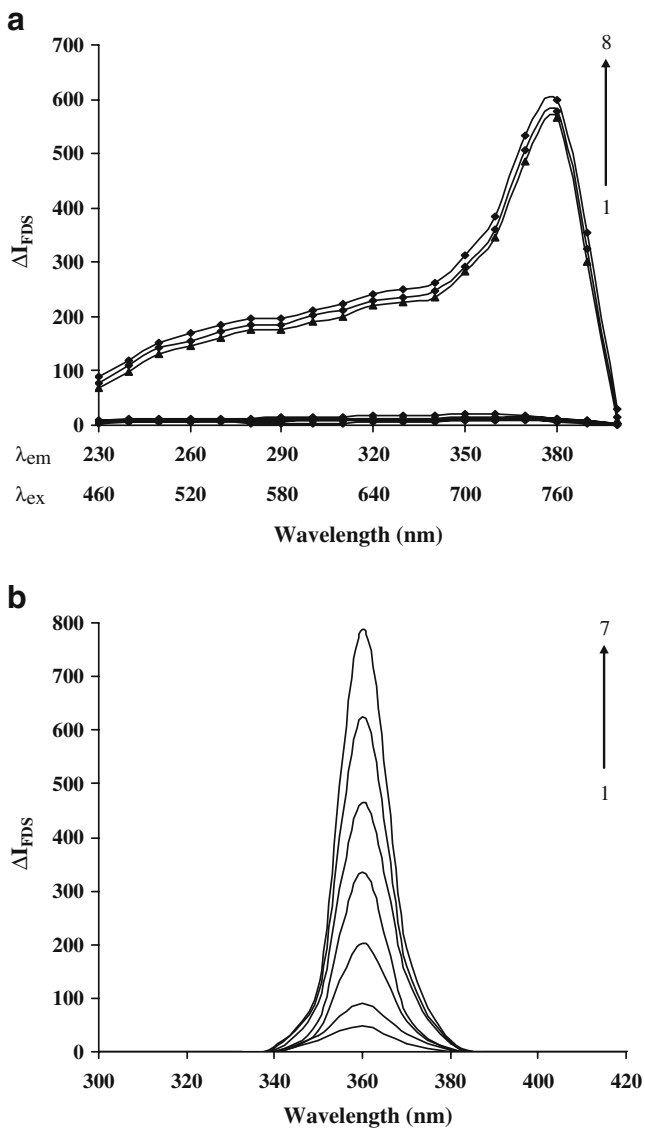


Fig. 4 (a) FDS spectra of FLD-Cu(II)-AS systems: From 1 to 8: (1) FLD, (2) Cu(II)-SDBS, (3) Cu(II)-SLS, (4) Cu(II)-SDS, (5) Cu(II)-FLD, (6) FLD-Cu(II)-SDBS, (7) FLD-Cu(II)-SLS and (8) FLD-Cu(II)-SDS. The concentration of CTRX: $2.5 \mu\text{gml}^{-1}$; the concentration of Cu(II): $2.5 \mu\text{gml}^{-1}$; the concentration of SDS and SLS: $3.0 \times 10^{-4} \text{M}$; the concentration of SDBS: $4.0 \times 10^{-5} \text{M}$ (b) FLD-Cu(II)-SDS system: From 1 to 7: the concentration of FLD: 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 and $4.0 \mu\text{gml}^{-1}$

The concentration of Cu(II) was kept constant at $3.0 \mu\text{gml}^{-1}$ in subsequent experiments.

Effect of the AS Concentrations

The RRS intensity enhanced gradually with increasing concentration of AS in FLD-Cu(II)-As system. When the concentrations of SDS, SLS, and SDBS were kept at $(1.0-5.0) \times 10^{-4} \text{M}$, $(2.0-5.0) \times 10^{-4} \text{M}$ and $(2.0-4.0) \times 10^{-5} \text{M}$, respectively, ΔI_{RRS} attained maximum (Fig. 7). If AS is not

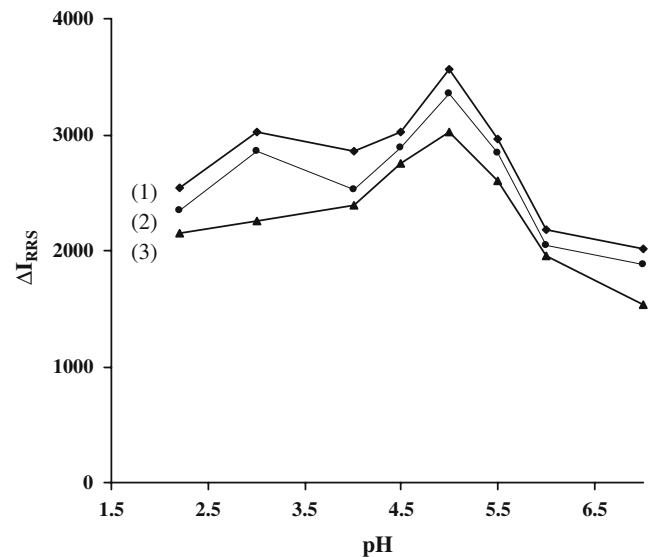


Fig. 5 Effect of the acidity: (1) FLD-Cu(II)-SDS (2) FLD-Cu(II)-SLS (3) FLD-Cu(II)-SDBS; The concentration of FLD: $2.5 \mu\text{gml}^{-1}$; the concentration of Cu(II): $2.5 \mu\text{gml}^{-1}$

adequate, the reactions would be incomplete but when AS is excessive, the intensities of RRS would decrease as a consequence of the formation of AS dimer by self-aggregation. The concentration of anionic surfactants were therefore, suitably adjusted.

Reaction Speed and the Stability of Scattering Intensity

The reaction completed in 10 min at room temperature and RRS intensity remained constant for 2.5 h at least.

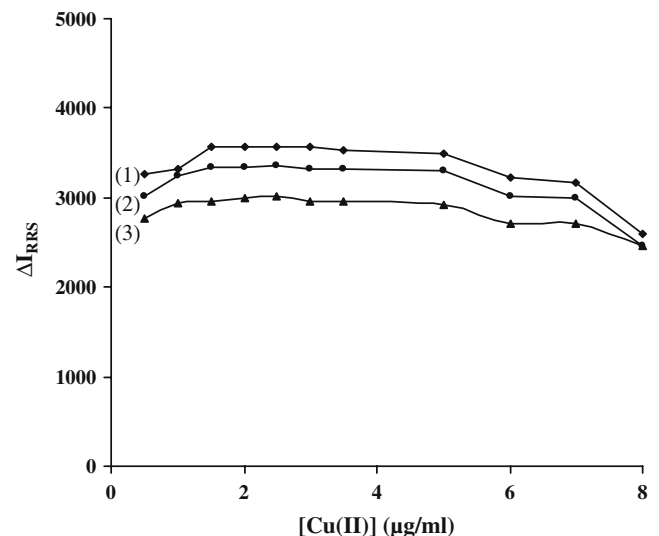


Fig. 6 Effect of the Cu(II) concentration: (1) FLD-Cu(II)-SDS (2) FLD-Cu(II)-SLS (3) FLD-Cu(II)-SDBS; The concentration of FLD: $2.5 \mu\text{gml}^{-1}$

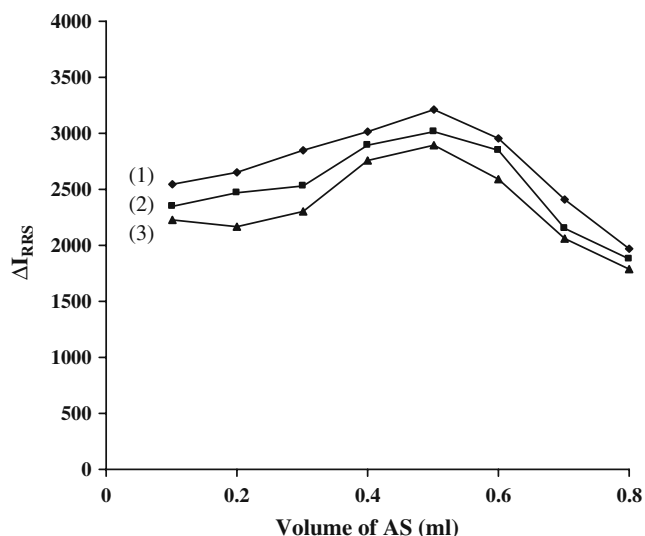


Fig. 7 Effect of the AS volume: (1) FLD-Cu(II)-SDS (2) FLD-Cu(II)-SLS (3) FLD-Cu(II)-SDBS; The concentration of FLD: 2.5 μgml⁻¹; the concentration of Cu(II): 2.5 μgml⁻¹; the concentration of SDS and SLS: 3.0 × 10⁻⁴M; the concentration of SDBS: 4.0 × 10⁻⁵M

Effect of Ionic Strength

The ionic strength was maintained by NaCl and its influence on the RRS intensity was studied. ΔI_{RRS} remained constant when the concentration of NaCl was kept below 1.0 × 10⁻²M, but above this concentration of NaCl, ΔI_{RRS} gradually decreased. The ion-association reaction occurs strictly under very low ionic concentration of NaCl.

Sensitivity of Method

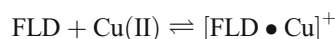
Varying concentrations of FLD was allowed to react with Cu(II)-AS in to form ternary complexes and their relative scattering intensities ΔI_{RRS}, ΔI_{FDS} and ΔI_{SOS} were

measured at their maximum scattering wavelengths after 10 min. The calibration graphs of ΔI_{RRS}, ΔI_{SOS} and ΔI_{FDS} against the concentrations of FLD were plotted (Table 1). It can be seen that the sensitivity of RRS method is higher than those of FDS and SOS though all these methods can be applied to determine FLD. The detection limits (3σ) of FLD by RRS method were 1.9 ng ml⁻¹ for SDS, 2.1 ngml⁻¹ for SDS and 2.2 ngml⁻¹ for SDBS systems, respectively. Although the sensitivity of all the methods were higher by one to three order of magnitude than those of common HPLC, flow injection spectrophotometry, spectrophotometry and fluorimetry. The RRS method had much higher sensitivity of all (Table 2). They proved to be more suitable for the determination of trace amounts of FLD.

Formation of Ternary Ion-Association Complex and its Effect on the Spectral Characteristics

The Ion-Association Reaction

Ratio of the ternary complex (FLD-Cu(II)-AS) was found to be 1:1:1 as established by Job’s method (Fig. 8). It can be formulated as [FLD•Cu(II)]AS. The reaction mechanism is depicted below:



The carbonyl group and nitrogen atoms on the prop- anamide appear to be most potential donor sites in FLD and expected to form a chelate through then [FLD•Cu(II)]⁺. Anionic surfactants (SDS, SLS and SBDS) at low concentration in weak acidic medium exist as monovalent anions SDS⁻, SLS⁻ and SDBS⁻, respectively. They can react with

Table 1 Analytical parameters for the determination of FLD

Method	Analytical reagent	Linear range (μgml ⁻¹)	Regression equation C, (μgml ⁻¹)	Regression coefficient (r)	Detection limit 3σ, (ngml ⁻¹)
RRS	Cu(II)-SDS	0.042–6.8	ΔI=358.6+868.4C	0.9951	1.9
	Cu(II)-SLS	0.052–3.5	ΔI=-14.9 + 1260.5C	0.9934	2.1
	Cu(II)-SDBS	0.059–2.9	ΔI=-189.4 + 1357.5C	0.9898	2.2
FDS	Cu(II)-SDS	0.41–5.9	ΔI=-45.1+134.8C	0.9974	2.3
	Cu(II)-SLS	0.49–5.5	ΔI=-55.4 + 130.9C	0.9924	2.4
	Cu(II)-SDBS	0.5–5.2	ΔI=-52.1+125.2C	0.9909	2.5
SOS	Cu(II)-SDS	0.28–3.5	ΔI=-1 9.0 + 322.0C	0.9940	2.4
	Cu(II)-SDBS	0.32–3.5	ΔI=-117.2+375.6C	0.9873	2.6
	Cu(II)-SLS	0.6–3.2	ΔI=-116.5+440.9C	0.9921	2.7

Table 2 Comparison of sensitivities of RRS method with other methods for the determination of FLD

Methods	Analytical reagent	Medium /condition	Linearity (μgml^{-1})	λ_{max} (nm)	LOD (μgml^{-1})	Literature
FI	SLS		100–400	310	0.21	17
HPLC	–	pH 4.0	2.9–11.6	240		18
Fluorimetry	–	–	0.1–0.6	$\lambda_{\text{ex}}/\lambda_{\text{em}}=255/375$	0.0086	19
SP	IDB	pH 12.0	0.4–16.0	570	0.131	20
	ANHD	pH 12.0	0.4–8.0	520	0.288	
RRS	Cu(II)–SDS	pH 2.2–7.0	0.042–6.8	345	0.0019	This work
	Cu(II)–SLS	pH 2.2–6.8	0.052–3.5	610	0.0021	This work
	Cu(II)–SDBS	pH 2.2–6.9	0.059–2.9	430	0.0022	This work

FI Flow injection spectrophotometry, SP Spectrophotometry, IDB Iminodibenzyl, ANHD 4-amino-5-hydroxy-2,7-naphthalenedisulphonic acid monosodium salt

$[\text{FLD}\cdot\text{Cu(II)}]^+$ to form ternary ion-association complexes through electrostatic attraction and hydrophobic forces.

Effect on RRS Spectra

It can be seen (Figs. 2, 3 and 4) that the formation of ion-association complexes can result in marked enhancement of RRS, FDS and SOS intensity. Since the sensitivity of RRS method is higher than those of others, it was used for the determination of trace amounts of FLD. The possible reason for RRS enhancement is given below:

Resonance Enhanced Rayleigh Scattering Effect When the wavelength of Rayleigh scattering is close to the molec-

ular absorption band, the scattering can absorb the light energy through resonance to produce a re-scattering process. As a result the scattering intensity is greatly enhancement [13, 14].

Enhancement of Hydrophobicity Since Cu(II), $[\text{FLD}\cdot\text{Cu(II)}]^+$ and AS^- are hydrophilic they easily dissolve and cannot form an interface with water. When $[\text{FLD}\cdot\text{Cu(II)}]^+$ reacts with AS^- to form the neutral ion-association complex $[\text{FLD}\cdot\text{Cu(II)}]\text{AS}$, a hydrophobic liquid-solid interface appears owing to the presence of the aryl framework of the ternary complex. This hydrophobic interface is helpful in the enhancement of RRS signal [15].

Enlargement of Scattering Molecular Volume The formation of ion-association complex enhanced the scattering molecular volume or weight, which also enhanced the scattering intensity. According to the Rayleigh-scattering formula [16]: $I = KCMI_o$ (where, I = resonance Rayleigh scattering intensity, I_o = incident light intensity, C = concentration of solution, K = proportional coefficient and M = molecular weight) When K , C and I_o are constant in Rayleigh-scattering formula, I is proportional to M . When binary chelate $[\text{FLD}\cdot\text{Cu(II)}]^+$ (M.W.=339.7) reacts with AS^- (SDS, SLS and SDBS) to form ternary complex $[\text{FLD}\cdot\text{Cu(II)}]\text{AS}$ the molecular weight increases from

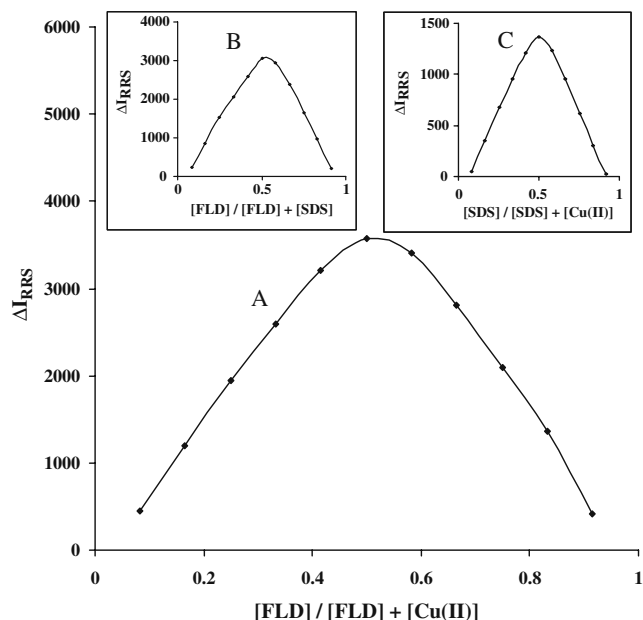


Fig. 8 The ratio of ion-association complex of FLD-Cu(II)-SDS system as established by Job's method of continuous variation: (A) SDS excess; (B) Cu(II) excess; (C) FLD excess

Table 3 Evolution of accuracy and precision of proposed RRS method

Added amount (μgml^{-1})	Found $\pm\%$ RSD	% Er	SD	% Recovery
0.05	0.49 ± 0.28	0.20	0.14×10^{-3}	99.80
0.50	0.505 ± 0.99	0.96	0.50×10^{-2}	100.96
1.0	1.016 ± 3.53	1.64	0.35×10^{-1}	101.64
2.0	2.024 ± 2.24	1.24	0.45×10^{-1}	101.24
4.0	4.021 ± 1.32	0.52	0.53×10^{-1}	100.52

Table 4 Result for determination of FLD in pharmaceutical samples

Method	Sample	Label claim (g per tablet)	Found (g)	Mean (g)	% RSD	% Recovery
RRS	Cytomid-250	0.250	0.251, 0.252, 0.251, 0.252, 0.249	0.251	0.487	100.40

339.7 to 628.08, 612.08 and 688.18. It is obvious that the RRS intensity is directly proportional to the molecular volume or molecular weight of the complexes.

Selectivity of RRS Method and its Analytical Application

Effect of Concomitant Substances

We investigated the effect of concomitant substances on the determination of FLD under optimum conditions. This method does not suffer any interference from commonly associated excipients and additives like sucrose, lactose, dextrose, starch, talc, stearic acid and sodium alginate. It is obvious that RRS method is highly selective.

Analytical Application

Determination of FLD in pharmaceutical sample Different amount of FLD was transferred into a 10.0-ml volumetric flask according to the general procedure. The % R.S.D (0.28–3.53) and % recovery (99.54–100.52) shows that the RRS method has a good repeatability (Table 3). Therefore, this method can be used for the determination of FLD in pharmaceutical formulation (Table 4).

Determination of FLD in urine A 1.0 ml aliquot of fresh urine (healthy human was filtrated and pipette into a 10.0 ml of volumetric flask for the determination of FLD according to general procedure. % RSD and % recovery were tasted by using the standard addition method the result was listed in Table 5. The results showed RRS method has a good repeatability. The % RSD and % recovery is between 0.44 to 0.89 and 99.9 to 100.3 respectively. Therefore, the method can be used for the determination of FLD in urine sample.

Table 5 Result for determination of FLD in urine samples by RRS method

Sample	Found amount (μgml^{-1})	Added amount (μgml^{-1})	Mean (g)	% RSD	% Recovery
Urine 1	ND	0.5	0.499	0.44	99.9
Urine 2	ND	1.0	1.003	0.62	100.3
Urine 3	ND	1.5	1.504	0.89	100.2

Conclusions

These methods for the determination of the FLD have been developed for the determination in pharmaceutical and urine samples. It is demonstrated that FLD reacts with Cu(II) and anionic surfactants to form ternary ion-association complex in 1:1:1 molar ratio. As a result, the RRS, SOS and FDS intensities of complex enhance remarkably. The increments (ΔI) are directly proportional to the concentration of FLD. These methods showed good repeatability and accuracy therefore they can use for the determination in pharmaceutical formulation, hospital and research laboratories.

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